

Original Article

METHANOLIC EXTRACT OF *BACOPA MONNIERA* REDUCE DEVELOPMENT OF TOLERANCE TO ANTIEPILEPTIC EFFECT OF PHENOBARBITONE IN MICE

VIJAYAKUMAR A. E.¹, VINAY M.¹, SEETHALAKSHMI¹

¹ESIC Medical College and PGIMSR, Chennai 600078
Email: aevijay1985@gmail.com

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ABSTRACT

Objective: Side effects and development of tolerance to antiepileptic drugs necessitate identifying alternative and improved approaches to treat epilepsy. *Bacopa monniera* (BM), is a natural product which is used to treat epilepsy. We evaluated effect of co-administration of BM with phenobarbitone (PHB) to prevent development of tolerance to PHB.

Methods: Male Swiss albino mice (25-30 gm-) were divided into six groups. Group 1 and 2 served as controls, which received vehicle and PHB (25 mg/kg, i. p.) respectively. In Group 3 maximal electric shock (MES) was induced two hrs following administration of PHB (25 mg/kg, i. p.) at alternate day for 18 d. Hind limb tonic extension (HLTE) occurrence on two consecutive days was set as end point. In Group 4, 5 and 6 MES was induced 2 h following co-administration of PHB (25 mg/kg, i. p.) and BM (250, 500 or 750 mg/kg p. o. respectively). Mice behavior tests were performed at the end of the 18th day, following which mice were sacrificed and brain was isolated for estimation of malonaldehyde (MDA) and reduced glutathione (GSH) levels.

Results: 82.5% of mice from group 3 developed tolerance to antiepileptic effects of PHB. Co-administration of BM (250, 500 and 750 mg/kg) lead to development of PHB tolerance in 82.5%, 25% and 20% respectively. This beneficial effect of BM was associated with reduced MDA levels and increased GSH levels in brain, suggesting a role of antioxidant pathways in preventing development of tolerance to antiepileptic effects of PHB.

Conclusion: Co-administration of BM prevented the development of tolerance to antiepileptic effects of PHB.

Keywords: *Bacopa monniera*, Phenobarbitone, Tolerance, Behavioral parameters, Oxidative stress.

INTRODUCTION

Epilepsy is a common neurological disorder affecting over 50 million patients globally [1] with an incidence rate of 2-10 per 1000 populations in the South Asia region [2]. Despite the availability of several antiepileptic drugs (AED), epilepsy can only be controlled and not cured, with surgery as an option in some difficult cases. Of concern are up to 30% of epileptic patients (medically intractable epilepsy or pharmaco-resistant seizures) in whom the seizures cannot be controlled even with the best available medications [3]. Phenobarbitone (PHB) is one of the most commonly prescribed AED in developing countries due to its efficacy and cost effectiveness. However, development of tolerance to antiepileptic effect of PHB is of clinical concern [4]. Resistance to antiepileptic drugs can be either primary or acquired. In primary resistance, the patient doesn't respond to the AED from the beginning of treatment and the disease persists in spite of regular drug therapy. While in acquired resistance, patients achieve initial positive therapeutic response but show recurrence of disease despite continuation of treatment.

Alternative medicine practice such as use of natural products constitutes a sizable part of global health care system. Many plant-based products are used in the management of epileptic seizures due to their cost effectiveness and relatively minimal side effects. *Bacopa monniera* (BM) (Family: Scrophulariaceae) commonly known as bramhi, found in India and Nepal is commonly used to treat CNS disorders. Bacosides A and B (a mixture of 2 saponins in BM), are reported to improve learning and memory and attenuate the retrograde amnesia produced by immobilization induced stress, electroconvulsive shock and scopolamine. Bacosides besides their antioxidant effects [5], enhance protein kinase activity and increase protein content in the hippocampus, which may lead to its memory enhancing effect [6]. High (close to 50% of LD₅₀) but not low (approaching 25% of LD₅₀) doses of BM extract given for 15 d demonstrated anticonvulsant activity [7]. Bacosides are reported to potentiate the therapeutic antiepileptic effect by reversing the alteration in glutamate receptor binding and NMDA R1 gene expression in the hippocampus of the temporal lobe of epileptic rats [8]. Hence in the present study we evaluated the effects of co-

administration of BM extracts on the development of tolerance to antiepileptic effects of phenobarbitone (PHB).

MATERIALS AND METHODS

Chemical

Bacopa monnieri (whole plant) plant extract was obtained from Natural Remedies Pvt Ltd, Bangalore, Karnataka, India. Methanolic extracts of *Bacopa monnieri* were prepared, concentrated using rotary evaporation and freeze dried until use. The profiling of plant extracts and a certificate of analysis was provided by Natural Remedies Pvt Ltd. The BM plant extracts had bacosides content of >40%. DMSO (Dimethyl sulfoxide) used as diluents. Phenobarbitone was obtained from Nichlos Piramal pvt Ltd Mumbai, India.

Experimental animals-Adult male Swiss Albino mice weighing 25-30 g were used in our study and kept in the animal house of AIIMS, Department of Pharmacology, Ansari Nagar, New Delhi, India. The animals were acclimatized for one week, prior to the experiments. All the experimental procedures were approved by the standing Institutional Animal Ethics Committee (IAEC) of AIIMS, New Delhi, India (AIIMS IAEC, No. 574/2010).

Experimental protocol

Albino mice were divided into six groups of six mice each. Group 1 served as vehicle control, group 2 received PHB (25 mg/kg, i. p.) for 18 d, group 3 received PHB (25 mg/kg, i. p.) with MES (50 mA, 299 pulse/s, 0.2 s) alternate day; Group 4, 5 and 6 received *Bacopa monniera* (250, 500 and 750 mg/kg, p. o.) respectively for 18 d along with PHB and MES treatment. *Bacopa monniera* dose selection was based on the previous study [7].

Maximal electroshock seizures (MES)

The MES model as described by Goodman & colleagues was used [9]. Briefly, before applying the Ear clip electrodes, the ears of the mouse were wiped with cotton soaked in normal saline. The shock was delivered using ECT unit (57800-001, UGO Basile, Italy). The frequency (299 p/s), current duration (0.2s) and pulse width

(0.9ms) were kept constant while the current intensity varied. The mice were observed for incidence latency and duration of hind limb tonic extension (HLTE). The mice were screened for presence of hind limb tonic extension (HLTE) in response to standard maximal electroshock (MES) challenge. Those mice responding with HLTE were used for further experimentation.

Phenobarbitone induced tolerance for MES mice model

Animals were treated with 100% protective anticonvulsant dose of phenobarbitone (PHB) i.e., 25 mg/kg/day i. p., 2 h prior to maximal electroshock [10]. Electroshock was repeated every alternate day while phenobarbitone was administered daily until the mice developed tolerance to PHB (evident from HLTE on two consecutive tests or 18 d whichever are earlier).

Effect of *Bacopa monniera* on tolerance

The mice were screened prior to the experiment and only those mice showing HLTE in response to convulsive stimuli were used for the study. Mice were co-administered PHB (25 mg/kg/day i. p.) and *Bacopa monniera* (250, 500 or 750 mg/kg/day, p. o) and 120 min later electroshock was administered and tested for development of tolerance to PHB as described above.

Parameters studied

Behavioral studies

In order to assess the effect of different treatment protocols on cognition in mice, three different tests were performed i.e., Closed field activity using actophotometer, Grip strength scores and Elevated plus maze [11].

These tests were assessed on initiation of protocol and at the end of protocol i.e. day 19.

Closed field activity test

Each mouse was observed for a period of 10 min in square closed arena equipped with infrared light sensitive photocells using a digital photo actometer (Techno, India Ltd.).

Grip test

The apparatus has a string of 50 cm lengths, pulled taut between two vertical supports and elevated to 40 cm from a flat surface. The mouse is placed on the string at a point midway between supports and evaluated according to the following scale: 0-fall off, 1-hangs onto string by two forepaws, 2-as for 1 but attempts to climb on string, 3-hangs onto string by two forepaws plus one or both hind paws, 4-hangs onto string by all fore paws plus tail wrapped around string, 5-escape.

Elevated plus maze

The apparatus consists of two open arms and two enclosed arms perpendicular to each other and is elevated from the ground. The mouse is placed on the open arm facing outwards and the transfer latency (TL) (the time in which the mice moves from the open arm to the closed arms) was recorded. On the next day, the mouse is placed similarly on the open arm and the TL is recorded.

Biochemical parameters

Oxidative stress

The oxidative stress markers, malondialdehyde (MDA) and reduced glutathione levels (GSH) were estimated in mouse whole brain tissue homogenate after 18 d in all 6 groups. The mice were decapitated under ether anesthesia and the brain was quickly removed, cleaned by rinsing with chilled normal saline and stored at -20 °C until analysis. The biochemical analysis was performed within 1 w. Malondialdehyde (an indicator of lipid peroxidation) and reduced glutathione were estimated as described by Ohkawa *et al.*, (1979) [12] and Ellman *et al.*, (1959) [13] respectively.

Statistical analysis

The data are expressed as mean±SEM. Results were analyzed using one-way ANOVA followed by Dunnett's *t*-test. *p*<0.05 is considered to be statistically significant.

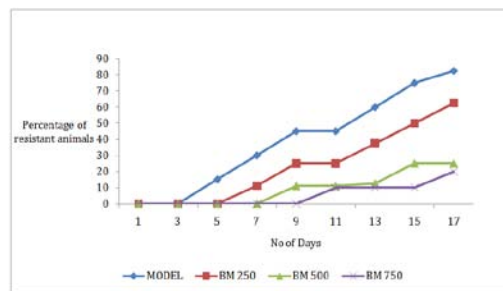


Fig. 1: Effect of *Bacopa monniera* on development of tolerance to phenobarbitone in mice

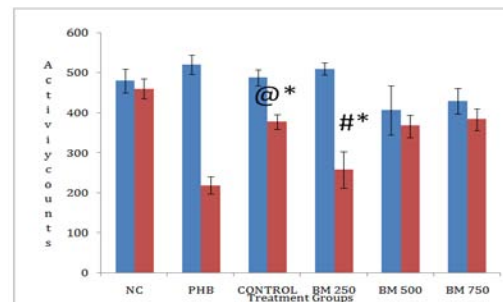


Fig. 2: Effect of *Bacopa monniera* on spontaneous locomotor activity using actophotometer in mice (10 min)

Each value represents mean±SEM for 6 mice
 @ p value for comparison with normal control group
 # p value for comparison with PHB+MES group
 * *p*<0.05; \$ *p*>0.05

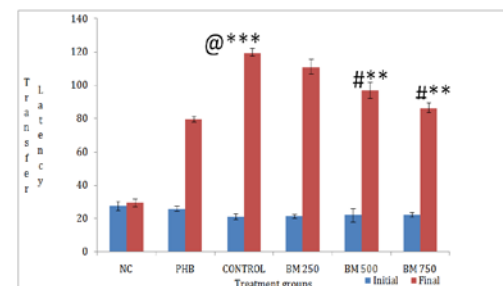


Fig. 3: Effect of *Bacopa monniera* on elevated plus maze in mice

Each value represents mean±SEM for 6 mice
 @ p value for comparison with normal control group
 # p value for comparison with PHB+MES group
 * *p*<0.05; \$ *p*>0.05

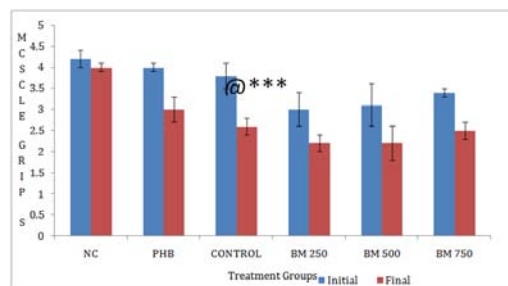


Fig. 4: Effect of different doses of *Bacopa monniera* on grip strength in mice

Each value represents mean±SEM for 6 mice
 @ p value for comparison with normal control group
 # p value for comparison with PHB+MES group * *p*<0.05; \$ *p*>0.05

RESULTS

Effect of BM on development of tolerance

At the end of the 18 d, 82.5% of mice from group 3 (PHB+MES) and 62.5% of the mice from group 4 (BM 250 mg/kg group) developed tolerance to PHB. However, in BM 500 mg/kg and 750 mg/kg, only 25% and 20% mice developed tolerance to PHB respectively (fig. 1).

Effect on behavioral studies

Effect on closed field activity

A decline in activity counts was associated with development of tolerance to PHB. This was evident in groups 3 and 4. Higher doses of BM 500 and 750 mg/kg, not only prevented the development of tolerance but also prevented decline in activity counts (fig. 2).

Effect on elevated plus maze

A significant increase in latency time was observed in tolerant mice and BM 250 groups in comparison to normal control suggesting

impairment of memory. While in BM 500 and 750 mg/kg groups significant reduction in latency time was observed as compared to tolerant mice suggesting that BM 500 and 750 mg/kg prevented the development of tolerance and collaterally improved cognitive function (fig. 3).

Effect on grip test scores

Statistically significant decline in grip test score was observed in tolerant mice however all BM administered groups had no effect on grip scores when compared to tolerant mice (fig. 4).

Biochemical estimations

Level of reduced GSH in resistant mice brain

A significant change was observed in the reduced GSH level between normal, control and tolerant group. BM at doses 250 and 500 mg/kg did not significantly alter the brain reduced GSH level in comparison to normal control and tolerant mice. At the dose of 750 mg/kg significant increases in brain reduced GSH levels were observed compared to tolerant mice (table 1).

Table 1: Effect of *Bacopa monniera* administered during development of tolerance to phenobarbitone on GSH&MDA levels in mice brain

Groups	GSH (nmol/g wet tissue)	MDA (μ g/g wet tissue)
Normal control	109.2 \pm 21.01	43.9 \pm 4.84
MES control	91.6 \pm 4.24	158.13 \pm 12.22
PHB+MES	68.5 \pm 9.58 ^{@***}	222.5 \pm 16.01 ^{@***}
PHB+MES+ <i>Bacopa monniera</i> 250 mg/kg	74.6 \pm 12.62 [#]	163.7 \pm 17.0 [#]
PHB+MES+ <i>Bacopa monniera</i> 500 mg/kg	80.3 \pm 19.27 [#]	151.3 \pm 23.28 [#]
PHB+MES+ <i>Bacopa monniera</i> 750 mg/kg	92.5 \pm 20.41 [#]	149.8 \pm 22.48 [#]

Each value represents mean \pm SEM for 6 mice

[@] p value for comparison with the normal control group, [#] p value for comparison with PHB+MES group, * p<0.05, *** p<0.001, ^{\$} p>0.05

Effect of drug resistance on level of MDA in mice brain

The level of MDA was significantly increased in mice developing tolerance to PHB as compared to a normal control group. Treatment with BM at all dose levels significantly decreased the brain MDA levels as compared to tolerant mice (table 1).

DISCUSSION

Pharmacoresistance is encountered in nearly one third of epileptic patient leading to consequences such as shortened life spans (higher incidence of sudden deaths), social disability owing to psychiatric issues and other AED related toxicities [14]. The Phenomenology of pharmacoresistance is complex with even controlled patients relapsing and eventually developing resistant epilepsy. While previously resistant patients would experience remission (about 4% of adult epileptic patients per year), of which a sizable proportion eventually relapse [15]. However, a subgroup of patients may have constitutional or inherent resistance. Although the treatment strategies for resistant patients may vary from curative or palliative surgery, procedures such as vagal nerve stimulation, ketogenic diet, these have limited utility and hence search is on for newer more effective AED [16, 17].

Many drug related factors have also been implicated in the development of pharmacoresistance. Of these, 'tolerance' is an important drug related factor [18]. Tolerance is reported to all AED [19] eventually leading to drug resistance. Hence strategies ameliorating tolerance development would prevent development of drug resistance at least in some patients. We tested once such approach in our study. The effect of extracts of *Bacopa monnieri* (BM) on the development of tolerance to antiepileptic effects of PHB was evaluated. Several studies have reported the anticonvulsant action [7] and learning and memory enhancement [20] effects of BM. Here it is also worthwhile to mention that BM has been used to treat various CNS disorders for a long period and is shown to be safe with very potent antioxidant activity.

Alteration in oxidative stress leading to pharmacoresistance is well reported, for instance increased production of ROS may modify ion channel properties and lead to pharmacoresistance. An over expression of MDR1 or P-gp in epileptogenic brain tissue of patients with different types of multidrug-resistant epilepsy, has been

demonstrated leading to the multidrug-transporter hypothesis of medically intractable epilepsy. Consistent with its profile, BM had a beneficial effect on oxidative stress parameters (MDA and GSH) in our study. Specifically the potential to prevent the development of tolerance to antiepileptic effects of PHB by co-administration of BM observed in our study is fascinating from multiple viewpoints. Firstly such approaches can be valuable in preventing an incidence of pharmacoresistance. Secondly co-administration strategies can lead to a reduction in the dose of the primary epileptic drug (in this case PHB) leading to reduce side effects associated with AED. Thirdly co-administration strategies can significantly improve therapeutics outcomes due to collateral benefits in improving cognitive functions on top of effectively achieving antiepileptic effects.

Effect of BM on tolerance

The mechanism by which BM prevented the development of tolerance to antiepileptic effects of PHB is although not clear. It could be speculated that the effects of BM on P-gp or antioxidant activity directly or indirectly via P-gp or its effects on ion channels, GABA channels and acetylcholinesterase inhibition may be involved. Nevertheless this warrants further investigation. Additionally cognitive and behavioral problems are of major concern in patients with intractable epilepsy; hence we analyzed the cognitive impact of BM using a battery of behavioral tests. Indeed impacting behavioral changes in rats were shown to be a predictor for pharmacoresistant epilepsy. We observed favorable effects of BM on behavioral parameters, which further supports, the co-administration of BM to achieve improved therapeutics effects of primary AED.

CONFLICT OF INTERESTS

Declared None

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